

Isolation of *Rickettsia heilongjiangensis* strains from humans and ticks and its multispacer typing

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INTRODUCTION

Tick-borne rickettsiosis in the far east of Russia was simultaneously observed in 1932 in two neighbouring territories: in the Primorye (Maritime) region by E. I. Mill and in the Khabarovsk region by N. I. Antonov and A. G. Naishtat [1,2]. The described illness was acute and febrile; skin manifestation presented with maculopapular rash, sometimes petechial. Typical epidemiology included tick bite 3–5 days before the onset of the disease. Based on these facts, Mill proposed a name of Primorye tick-borne fever; studying the same problem independently, Antonov and Naishtat proposed to call it Far-Eastern tick-borne typhus. Later, in 1935–1939, a similar disease was found in central Siberia in the Krasnoyarsk region. The aetiology of the disease remained unknown, although rickettsial origin was evident; the researchers assumed that agents of ‘tropical tick typhus’ or scrub typhus might be responsible for both diseases, in the Far East and Siberia. Beginning in 1938, several expeditions have been organised in Siberia and in the Far East under the leadership of M. K. Krontovskaya. The disease has been found in both regions; geographical distribution associated with specific tick vectors (*Dermacentor* spp. in Siberia; *Haemaphysalis concinna* and *Dermacentor silvarum* in the Far East) and mammal reservoirs was identified. The aetiology of the disease was studied by O. S. Korshunova, who had isolated the first strains of the agent of tick-borne typhus in the Far East and Siberia. Later on, the disease in both regions (Siberia and the Far East) was considered to be

caused by the same agent isolated in Siberia and called *Rickettsia sibirica* [3].

An attempt to discover the aetiology of tick-borne rickettsiosis was made in the Russian Far East in 2004 [4]. Surprisingly, in all patients, instead of suspected *R. sibirica*, another agent, recently (1982) isolated in northern China, *Rickettsia heilongjiangensis*, was discovered. Although it was suspected to be an agent of human disease, no evidence was published and the only known strain was isolated from ticks. Recently, molecular identification of a rickettsial collection showed that one of strains isolated from ticks in 1966 in the Altai region is also *R. heilongjiangensis* [5].

MATERIALS AND METHODS

Seven patients hospitalised in 2006 in the infectious diseases department (Khabarovsk, Russian Far East) with clinical and epidemiological signs of spotted fever group rickettsiosis, were studied. All patients presented with eschars. They did not receive any antibiotic treatment before specimens were taken and the duration of the illness varied from 4 to 7 days. Whole blood (0.5 mL) and well-ground eschar biopsies (approximately 50 mg) were mixed and inoculated intraperitoneally in young (15–20 g) male *Phodopus* hamsters. Rectal temperature was measured in hamsters twice a day before sacrifice. Sacrificed after 7 days, unopened hamsters were deep frozen (–80°C), stored and transported during 3 months. Alive *Haemaphysalis concinna* ticks were collected from wild vegetation (30) and patients (1). Suspensions of four defrozen hamster organs (brain, spleen, liver and tunica vaginalis) and ground ticks were shell-vial centrifuged with a monolayer of L929 cells. Isolated strains were identified by PCR/sequencing with common rickettsial gene primers (*gltA*, *sca4*). Three rickettsial spacers were chosen for typing: *dksA-xerC*, *rpmE-tRNAmet* and *mppA-purC*. Fragments were concatenated, aligned and a UPGMA phylogenetic tree was built on MEGA3 software [6].

RESULTS

Beginning from day 4, a slight (up to 38.5°C) decrease of temperature was noted in six animals, from which the strains were later isolated. Visual signs of infection included languor and

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No conflict of interest declared.

decreased appetite. No scrotal phenomena were noticed.

Six bacterial strains were isolated: four from humans and two from ticks, collected from humans and vegetation. Microorganisms grew intracellularly and showed typical rickettsial morphology when coloured by Gimenez staining. PCR for all strains produced positive results and all strains were identified as *R. heilongjiangensis* by sequencing of amplified DNA fragments. Phylogenetic analysis based on aligned concatenated spacers (multispacer typing) showed identity of all six strains isolated in the Khabarovsk region and its visible difference from the prototype strain HLJ054 (Fig. 1).

CONCLUSIONS

The authors have isolated the first human strains of *R. heilongjiangensis* in Russia and confirmed the aetiology and vector of tick-borne rickettsiosis in the Russian Far East. No evidence of *R. sibirica* human infection in the Russian Far East was found. Based on data collected from 2001, the authors conclude that tick-borne rickettsiosis cases in the Russian Far East are caused by *R. heilongjiangensis* and propose that the previously unnamed disease be called Far Eastern tick borne rickettsiosis. Moreover, as the first rickettsiosis in Russia and Asia was described in the Khabarovsk region (Russian Far East) in 1934–1936, it probably was the same disease caused by *R. heilongjiangensis* and called nowadays Far Eastern tick-borne rickettsiosis, so it may be

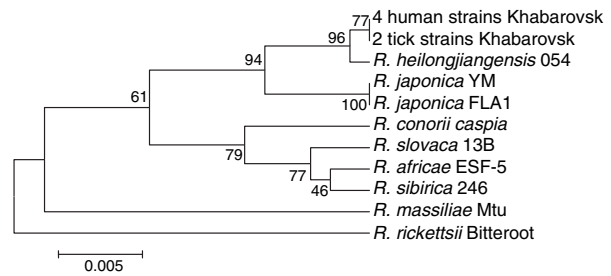


Fig. 1. UPGMA tree constructed with concatenated sequences of spacers *dksA-xerC*, *rpmE-tRNAmet* and *mppA-purC* without ambiguous sites and tandem repeats.

called the first tick-borne rickettsiosis described in Asia.

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